V.

CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY.

XV. — ON THE STRUCTURE AND DEVELOPMENT OF CHOREOCOLAX POLYSIPHONIÆ, REINSCH.

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HERETOFORE comparatively little has been known concerning the obscure parasitic alga, *Choreocolax Polysiphoniæ*, Reinsch. Little has been written concerning it, and, so far as I know, only one other of the various forms included in the original description under the same genus has been the subject of even a note. It was with a hope of adding something new to our knowledge of it that I undertook the examination of this plant. The observations resulted in the discovery of several interesting facts, which, together with a general description of the alga are embodied in this paper.

The literature concerning the genus Choreocolax, besides the original description, consists of only a few scattered notes. The genus was first described and figured by Reinsch in his "Contributiones ad Algologiam et Fungologiam." * There he mentions several species parasitic on various algæ, but described only from sterile specimens. Among them is *Choreocolax Polysiphoniæ*, growing on *Polysiphonia fastigiata*, the only species that has been reported to have been found on the American coast since Reinsch's original descriptions. The next notice of these parasites is by Farlow, in his New England Algæ,† where in a foot-note he briefly mentions them. In a paper published later, ‡ he makes mention of *C. polysiphoniæ*, describing for the first time the tetraspores of this plant. They develop from the terminal cells of the plant, and may be either tripartite or cruciate ; usually the

* Page 61, Plate XLIX.

† Page 101.

‡ On some New or imperfectly known Algæ of the United States. Bull. Torrey Bot. Club, Vol. XVI. No. 1, p. 6, Plate LXXXVII.

latter. Recently Reinke and Schmitz found in one of the species formerly described as *C. mirabilis* cystocarpic fruit which enabled them to ascertain its true affinities. They placed it among the Gelidiaceæ, and gave to it the new generic name of Harveyella.* Their reasons for placing it in a new genus were twofold. They rightly thought *C. mirabilis* to be widely different from *C. Polysiphoniæ*. Besides this, *C. Polysiphoniæ* was described before *C. mirabilis* in Reinsch's original account, so that the former is to be regarded as the type of the genus Choreocolax rather than the latter. Besides these notes, Choreocolax and Harveyella are scarcely more than mentioned by name in a few other places. Schmitz in his arrangement of the genera of the Florideæ † places them both among the Gelidiaceæ. Batters mentions them also in his List of Berwick Algæ, ‡ and reports the collection of tetrasporic specimens of *C. Polysiphoniæ,* but has nothing new to add regarding them. Thus it will be seen that the literature concerning these interesting parasites is very scanty.

Choreocolax Polysiphoniæ grows, as has been already stated, upon *Polysiphonia fastigiata,* a common alga along the Northern New England coast. The parasite was sufficiently abundant at Nahant, Mass., to be collected in considerable quantities from the middle of November to the latter part of the following March. I found it also at Newport, R. I., growing on the Polysiphonia, on the more exposed points. *C. Polysiphoniæ* has been collected by Dr. W. A. Setchell at various points along Long Island Sound, though its host is much less common there than farther north. Mr. F. S. Collins has sent me specimens he found at Mount Desert, on the Maine coast, during the month of July, 1890. From all reports, however, it is found nowhere in such quantities as at Nahant. Batters § mentions *C. Polysiphoniæ* as having been collected on the British coast at Berwick Bay, but adds that it is rare. The distribution of this alga, then, is fairly wide, and it is probable that wherever its host is found it may also be expected in greater or less quantities.

To the naked eye the fronds of Choreocolax appear as small whitish brown dots of variable size, situated almost always in the dichotomies of the Polysiphonia. In some specimens collected at Nahant, almost every axil except the ones of the very youngest branches was

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* Algenflora der west. Ostsee. Deutsch. Anth., Kiel, 1889, p. 28.

† Systemat. Uebersicht der bisher bekannten Gatt. der Florideen. Flora, 1889, Heft V. p. 439.

‡ Pages 126 and 142.

§ List of Berwick Algæ, p. 142.

occupied by a frond of the parasite. Such cases as these, however, are not usual; ordinarily the host is not so completely covered with the Choreocolax. That the parasite has a deleterious effect on the Polysiphonia is very evident, for the fronds of the latter, on which the Choreocolax is most plentiful, are always paler and less vigorous-looking than the fronds not so affected. The growing tips of the host plant, which usually give every appearance of active growth, are fewer in number, small, and often distorted. Often the terminal branches can be seen to have shrivelled up and rotted away, probably from insufficient nourishment. In fronds of Polysiphonia, all stages of this decay may be seen, which varies in intensity according to the amount of Choreocolax on the frond. Some exceptionally strong plants seemed little affected, though considerably attacked by the parasite.

On examining specimens of *Choreocolax Polysiphoniæ* with a hand lens, they are seen to be usually light-colored masses varying in shape from a flattened hemisphere to almost a sphere. The surface of most of them is smooth and the outline of the frond is regular (Fig. 1), though some are divided unevenly into several lobes (Fig. 2). The cause of this latter condition will be discussed subsequently. The size of these masses that constitute the external portion of the Choreocolax is very variable, ranging from small spots that can scarcely be seen, even with a powerful hand lens, to bodies about 2 mm. in diameter. The majority of them, however, are not over 1.5 mm. in diameter. The small fronds are flattened, becoming more and more nearly spherical as they increase in size and age. It is only the large fronds that are lobed in the manner mentioned, the small ones are always quite regular in shape. The color of the fronds varies from the translucent whitish color of the young ones, to the dirty reddish appearance of the adult specimens. Occasionally the latter are dark brownish red, though usually they are not very deeply colored, and may sometimes be almost as white as the young fronds. The larger masses of *C. Polysiphoniæ* are of a tough cartilaginous consistency, firmer and more unyielding than the more gelatinous younger fronds. The appearance of *C. Polysiphoniæ* is so unique, that, together with its habitat, the collector is easily informed of the identity of this rather insignificant-looking alga.

Before describing the growth and development of the frond, it will be best to explain the structure of the adult plant, which cannot be well compared with the conditions presented by other algæ. A section through the frond shows it to be composed for the most part of large, somewhat irregular cells, approaching a spherical or cylindrical shape, which are filled with coarsely granular, almost colorless contents. The cells are separated, except at narrow points of contact, by an almost structureless gelatinous intercellular substance (Fig. 8). This gelatinous substance, which contains a large amount of water, is found to a greater or less extent between all the cells of the frond, and gives to it the consistency already mentioned. The large cells which make up the interior of the frond are not at all regular in either size or shape, some departing so far from the spherical as to become almost branched, by the excessive growth of some portion of the cell in some other direction than that of the main axis. They are not arranged in filaments, or in any distinct order, but are joined in a loose parenchymatous network. In the parts of the frond near its point of attachment to the host plant, the cells are seen to be smaller and of a different shape than those of the rest of the plant. They are cylindrical, with one axis considerably longer than the others, and are arranged in filaments of greater or less length (Figs. 8, 14). The filaments which may or may not branch, make their way beneath the peripheral siphons of the Polysiphonia, encircling its axial row of cells. Usually these filaments extend from the frond of Choreocolax from which they arise, through the length of three or four cells; cases were observed, however, where they had penetrated as many as ten cells from their starting point. It is by means of these cells that the Choreocolax obtains elaborated material from the Polysiphonia, on which it depends, in a large measure at least, for its nourishment. The close connection which the filamentous cells of the parasite have with the cells of the host may be easily demonstrated. A section shrunk in glycerine and stained with Hofmann's blue, enables one to see with the greatest distinctness threads of protoplasm connecting the cells of the two plants (Fig. 7). Material killed in osmic acid also shows this point to advantage. At the same time it will be seen that the cells of the Choreocolax attach themselves almost wholly to the cells of the central siphon, although sometimes the walls of the peripheral siphon are penetrated and the material afforded by them appropriated by the parasite. *C. Polysiphoniæ* is then, as Reinsch first maintained, a true vegetable parasite, which depends in the main for its nourishment on the materials provided by its host, exerting upon the latter nothing but a deleterious influence. These filaments were never seen to connect with any of the external swellings except the one from which they arose. Each swelling then represents a separate frond, and there is no continuous growth of filaments which ramify through the host, rising at places in external prominences for the purpose of producing fruit. In the external portion of the frond, the proportion of the filamentous cells to the globose ones is very variable. Sometimes the former encroach far on the latter, while again the globose cells may entirely exclude the filamentous ones from the external frond. In the same way, the size of both kinds of cells varies a great deal; in fact, in all the structures of the frond, even in rare cases in the fruit, a great diversity in appearance may be noticed.

Besides the kinds of cells already described, the peripheral cells of the frond present a very characteristic appearance. Nearer the outside the cells are seen to be smaller, more nearly spherical in shape, and more regularly arranged, than in the rest of the frond. The gelatinous intercellular substance is also considerably diminished in quantity in this region. Those cells which form the extreme outer layer are still different in shape from any of the others. They are somewhat elongated and pyriform, the smaller ends being directed inwards (Figs. 5, 8). They constitute the growing part of the frond, as will be described later, in discussing the development of the plant. The contents of these outer cells is more granular than that of the others, and the nuclei are more distinct; in fact, they present all the appearances characteristic of growing cells. Directly outside of the pyriform cells there is a thick sheath of cellulose, which covers and protects the whole frond (Figs. 8, 17). This outer skin of cellulose is not formed by the fusion of the exterior walls of the peripheral cells; they are only loosely connected with it, and may be detached from it without injury. The cellulose sheath may be dissected off in large pieces, when it is seen to be almost structureless, except for the depressions left by the cells which had formerly been attached to it, and for the irregular blotches of brownish red coloring matter in it. By this means the pigment to which the color of *C. Polysiphoniæ* is due may be seen to be contained almost entirely in this external covering. Sometimes the peripheral cells may be also tinged with brown, while in one or two cases the whole tissue of the frond partook of this color. The sheath is nothing more than a thickening of the gelatinous intercellular substance on the outside of the frond. This gelatinous substance is itself but a modification of a portion of the walls of the cells, and gives a cellulose test with chloriodide of zinc as well as the sheath. As in the case of the other cells of the frond, those near the periphery are subject to some variation. In those fronds where the filamentous cells extend into the external protuberance of the frond, the peripheral cells partake more or less of the same character. One extremely exaggerated case was noticed, in which the cells around the circumference of the frond were enormously elongated. This frond happened to be tetrasporic, and the tetraspores were also greatly elongated and deformed. The contents of the cells present little of interest. The outer cells are filled with a very highly granular protoplasm, in which the nucleus is very conspicuous. The inner ones contain less of the granular protoplasm, and the nucleus is rather more indistinct. All the cells of the plant are usually colorless, though sometimes they may be tinged with purple, especially the filamentous ones in closest contact with the Polysiphonia. Of ordinary starch there is no trace to be found, iodine giving only a deep brown color to the whole of the contents of the cells. The walls of the cells are cellulose, and are not remarkable. One interesting feature regarding the cells of *C. Polysiphoniæ* is their great variability in the amount of contained food material. This varies from the condition found in the globose cells of the young plant, which are gorged with protoplasm, to the decidedly contracted and starved appearance presented by the cells of some of the adult plants. This latter condition is particularly to be noticed in the tetrasporic fronds which are almost ripe, the growth of the tetraspores having apparently taken all the food material held in reserve by the plant. Figure 8 shows a tetrasporic frond where the cells are somewhat affected, and is a good example of the condition of the average frond. In Figure 14 the cells are seen to be well gorged. The difference in appearance is often so striking as to lead one to think at first sight that plants are not of the same species.

The development of the frond I was able to follow with considerable certainty, except in the youngest stages, of which fewer specimens were found. As has been said before, the fronds of *C. Polysiphoniæ* are almost always found in the dichotomies of the host plant, and the reason for this can be explained by the following circumstances. In the axils of the branches of *Polysiphonia fastigiata* there is often collected more or less organic or inorganic material, and they are also frequently occupied by some of the many epiphytic algæ that grow upon this plant. Besides the natural shelter afforded by the axils, these growths enable the spores to become attached before they are able to make their way through the tissues of their natural host. The spore becomes buried in the organic matter collected in the axil, and in this position begins to germinate. The earliest stage of a developing spore of Choreocolax that was found was one where five cells were to be distinguished. The spore had apparently divided into four parts, and then after some growth one of these four cells had divided again into two, — a process which the other cells were probably about to undergo. There is little to be said regarding this; what nourishment the young frond required to carry on the growth was probably taken from the organic material in which it was buried. No growth had yet penetrated the cells of the Polysiphonia.

The next stage that was observed in the development of the frond was rather more complicated. The young frond was here composed of a considerable number of cells, which, however, presented as yet no very definite arrangement (Fig. 3). The gelatinous intercellular substance was present to some extent, and a tougher layer of it already covered the outside surface. The first indications of the growth of the parasite into the Polysiphonia were also seen here. In the figure (Fig. 3) where this stage is shown, two cells will be seen that have thrust themselves between the cells of the host plant, and have grown some little distance inwards. Even as early as this the young frond of Choreocolax must have obtained some nourishment from the Polysiphonia, or it would not have given evidence of so much activity of growth. Other than this there is no differentiation in the cells of the frond; the characteristic arrangement of the terminal layer that is developed in the adult frond has not yet made its appearance. Having once forced their way into the tissue of the Polysiphonia, the cells of the Choreocolax grow more rapidly, and finally come to encircle cells of the host plant. New filaments push their way in, and grow in both directions, between the central and peripheral siphons of the Polysiphonia, attaching themselves chiefly to the former.

In the mean time the external portion of the frond has been increasing in size. The cells which have pushed themselves into the host plant have, besides fastening themselves to its cells, begun to send out branches upwards, which, by subsequent growth, are to form a part of the external protuberance of the frond. As these cells increase in number they press outwards, and, joining with the rapidly developing external portion already formed, displace the cells of the host plant in the immediate neighborhood of this growth. Later, the displaced cells of the Polysiphonia are entirely enveloped by the growing Choreocolax. No morbid growth is stimulated in them, however; they remain entirely passive, and are gradually absorbed by the parasite, so that in adult specimens there is usually no trace of them left.

Comparatively early in the development of the frond, before the internal growth of the vegetative filaments has pushed aside the cells of the Polysiphonia to any great extent, it will be seen that the peripheral cells of the external portion of the Choreocolax frond present an appearance different from those in the interior. They have become arranged in a regular layer one cell deep over the entire surface of the frond, covering the more or less promiscuous mass of cells beneath (Fig. 4). The internal cells divide and grow to some extent, but it is from the outer, regular layer of cells that the larger part of the exterior portion of the frond is to be developed. The growth inside of the host plant also helps in the formation of this part of the frond, but it is only for a short time that it can be distinguished from the growth of the peripheral cells just mentioned. As soon as the cells which arise from the inner filaments make their way between the cells of the Polysiphonia to the outside, they become arranged in this regular order and blend with the rest of the frond, becoming indistinguishable from it. Cases have been seen where, owing to irregular growth, they did not unite; and then, instead of one large protuberance, there were many smaller swellings closely bunched together. The surface of the young frond, at first almost a plane, becomes rapidly convex by the more active growth of the cells in the centre of the frond. Finally, the hemispherical or almost spherical mass is formed in which the fruit is later borne.

The ordinary method of growth of the frond in distinction to the manner of development in its earlier stages is now to be considered. It is essentially the same after the condition is reached where the peripheral cells are arranged in a distinct layer. Before that time the growth is irregular and unequal. Taking a single peripheral cell and following its growth throughout, we find the method to be as follows. First, the cell is divided into two parts by the formation of a transverse wall. The lowest half of the cell does not divide again, but merely increases in size. The upper cell, on the other hand, is soon divided in two by the formation of a vertical wall, and these two cells ultimately become four by the division of each into two, in a vertical direction at right angles to the first vertical division (Fig. 6). The four cells thus formed repeat the process of division first described, and by this means the frond is enlarged in all three dimensions. The number of cells into which the outer row of cells may divide vertically is not necessarily four. There may be only three, or sometimes as many as five cells so formed, but the important point is that they are equally distributed, so that, besides extending the frond vertically, they increase it almost equally in both of the other directions of space. Other irregularities are also noticeable; sometimes the transverse division fails to take place in some of the cells, and leaves a conspicuously long and ill-shapen cell, which, however, continues its growth like the others. At the time of the most rapid growth the formation of the walls follows so quickly on one another that the newly formed cells do not reach their full size before they divide again. As a consequence of this the outer portion of an actively growing frond is made up of groups of small, closely compacted cells, each group having originated from the division of a single terminal cell (Fig. 5). The cells of these groups gradually grow and assume the normal appearance, the outer ones continuing to divide, though more slowly than before, and the inner ones losing themselves in the inner mass of the frond. The activity of the terminal cells almost entirely ceases as the frond approaches maturity, and in the adult frond there is no sign of further growth.

The tetrasporic fruit of Choreocolax was, as has been said at the beginning of this paper, first mentioned by Farlow, who gives a brief account of it. The tetrasporic fronds were not uncommon in the material I collected at Nahant, and material was easily found from which to study them. They were no more frequent at one time than at another, during the portion of the year in which I looked for them. Externally, the tetrasporic plants present no characters by which they may be invariably distinguished from sterile specimens. The size of the frond bears very little relation to the presence of even mature tetraspores, for it is not at all unusual to find in a very minute frond not a millimeter in diameter tetraspores which are to all appearance perfectly developed. A vertical section of one of the hemispherical swellings shows the tetraspores to be located on the extreme periphery of the frond (Fig. 8). There is no definite limit to the number of tetraspores to be found in a single specimen; sometimes there are very few of them, while at other times there are so many that they have quite crowded the terminal cells out of place. All stages of growth of the tetraspores are present in one frond at the same time, so that their development is not hard to trace. They arise from the enlargement of certain of the terminal cells, but there is no criterion by which it is possible to tell what ones will develop into tetraspores. The first indication is a slight swelling of those cells which are to form the spores (Fig. 9). They rapidly increase in size, the contents of the transforming cells at the same time taking on a more granular appearance than their unmodified neighbors (Fig. 10). After the single cell has attained almost the size of the mature spore, a transverse wall is formed across it (Fig. 11), and is soon followed by a vertical one, which thus divides the spore into four parts, producing a very typical cruciate tetraspore (Fig. 12). Sometimes in the two-celled stage the longitudinal division of the distal cell precedes that of the proximal one, giving the spore the appearance of being tripartite. The longitudinal wall of the proximal cell is ultimately formed, however, and then the spore presents the usual cruciate aspect. True cases of tripartite spores are to be found, however, where the longitudinal division of the lower cell has actually taken place in a direction at right angles to that of the upper one (Fig. 13). The contents of the tetraspores do not differ very markedly from those of the other cells, except that they are more highly granular. Fully adult spores from fresh specimens are usually of a brownish color, and measure on the average $45.5 \times 28\,\mu$. Some apparently mature ones were much smaller, being only $25 \times 18\,\mu$. The curious case of distortion of the tetraspores where they were so enormously elongated has already been noted; they measured about 80 μ long by 15–20 μ broad. The tetraspores make their way out by the breaking away of the outer cellulose skin, which becomes very weak as the frond increases in age, and may then be easily ruptured by slight pressure. Attempts were made to germinate the tetraspores, but all proved unsuccessful. It may have been that the conditions were unfavorable, or perhaps that the tetraspores rest some time before germinating.

Besides the tetraspores, no one, so far as I am aware, has ever given an account of the reproductive organs of *Choreocolax Polysiphoniæ.* When it was found that the non-sexual reproduction of *C. Polysiphoniæ* was by means of tetraspores, this alga could be classed with much more certainty among the Florideæ, and it was reasonable to suppose that cystocarps might be found on further search. It was, indeed, with this possibility in view that I was led to investigate *C. Polysiphoniæ*. In all of the material collected during the fall and early winter of 1890, nothing but the tetrasporic fruit was noticed. Some specimens obtained at Nahant, on December 11th, proved more interesting. In a few of the fronds, structures were found which at once appeared could be nothing else than cystocarps. These observations were corroborated later, and more cystocarpic material was procured, which enabled me to make out definitely the structure of the fruit, and to some degree also its development. It was not until much later that the trichogynes were first seen, and as it was not possible to trace out the complete course of development from the trichogyne to the ripe cystocarp, it will be best to begin with a description of the latter.

The ripe cystocarpic fronds can usually be distinguished from the others by the fact that they are more or less lobed, each lobe containing a single cystocarp (Fig. 2). This is not a very reliable distinction, however, for when a frond contains only one cystocarp, which not infrequently happens, its shape closely resembles that of a tetrasporic or sterile frond. On the other hand, the other fronds are sometimes lobed, from abnormal conditions of growth, in manner not unlike the cystocarpic specimens. Thus it will be seen that it is impossible to tell certainly, without microscopic examination, in what state any particular frond may be. In the majority of the cystocarpic fronds there are several — from two to five — cystocarps present, though a considerably larger one is, as has already been said, often found in place of many.

Although it might seem to indicate, from the division of the frond into lobes, that the cystocarps are in this instance external, closer search shows that this cannot be considered to be the fact; the growth of so large a body as the cystocarp in so small a frond naturally necessitates the condition found, and even as it is the lobes represent more than merely the conceptacles themselves, for the ordinary tissues of the frond go in part to make them up (Fig. 14). The cystocarps are ovoid to almost spherical in shape, with the smaller end external. They may be readily separated from the surrounding cells by a little careful dissection, when they appear as small white dots, scarcely visible to the naked eye. From a vertical section of a cystocarp a very good idea of its structure may be obtained (Fig. 14). The cells surrounding the cavity in which the spores are borne are seen to be more closely compacted than those of the rest of the frond, and of a different shape. This closely compacted wall consists chiefly of sterile cells, with which on the inner surface the spore-bearing cells are intricately associated. The conceptacular wall is always thickest at the inner end of the cystocarp, gradually becoming thinner as it approaches the outside, being represented in the region of the carpostome by a single layer of cells. The carpostome, which has always been seen in these cystocarps, consists of a small circular opening through the cellulose covering of the frond. It is situated at the small end of the cystocarp, where it approaches nearest the exterior of the frond. The cells which compose the wall of the cystocarp, when viewed in vertical section, are seen to be either spindle-shaped or very thin and almost filiform (Fig. 14). This is due to the collection of the protoplasm of the cell at the centre, leaving but a small amount at the extremities. Other aspects show the cells to be flat or tabular, with often a very irregular outline. They may then, in a general way, be said to be of a lenticular shape, although they do not often approach the circular in form. They always lie with their flattened faces presented to the interior surface of the cystocarp, and consequently a vertical section, if it be not tangential, always exhibits the cells in their fusiform appearance. The amount of gelatinous intercellular substance between these cells is much smaller than is found elsewhere, except perhaps in the growing terminal part of the frond.

It is on cells very like the ones just described, perhaps somewhat thicker in proportion to their other dimensions, that the carpospores are borne. The cells from which the spores arise lie directly inside of the conceptacular wall, and are, as has previously been said, closely interwoven with it. They have no peculiarities in shape or structure which distinguish them from the sterile cells forming the wall. They do not, however, like the latter, always present their flat faces inwards, but are more irregularly arranged. The spores are borne from the ends or angles of the cells, or from protuberances arising from their flat surface (Figs. 15, 16). The entire surface of the cavity is lined with the spores, except in the immediate neighborhood of the carpostome (Fig. 14). They are not arranged in chains, but are borne singly. In shape they are irregularly ovoid or pyriform, with tapering and sometimes acute apices. A small basal cell is always found between the spores and the spore-bearing cells proper. From the basal cell there arises a stèrile filament that always appears to be present (Figs. 15, 16). This paraphysis is usually somewhat longer than the spore, but as it arises from the side, and not the top, of the basal cell, it extends out no farther. Its contents are almost hyaline, in contrast with those of the spore. In the mature cystocarp the spores are directed inwards, and somewhat upwards, towards the carpostome, almost completely filling the cavity. The ripe spores are highly granular, somewhat darker in color than the other tissues of the plant, and have distinct nuclei. That the cystocarps examined were ripe, there can be no doubt. Some specimens collected on December 23d were placed uninjured in sea-water on a slide; when they were examined, some fifteen hours afterwards, many spores were found to have made their way out of the cystocarp, and to be lying loose in the surrounding water. Attempts were made to germinate the carpospores as well as the tetraspores, all of which failed, owing, no doubt, to the same causes suggested in the case of the tetraspores.

Before leaving the subject of the cystocarps, it is necessary to describe a peculiar condition of the frond that was always found in cystocarpic plants. In the peripheral portion of such a frond, instead of finding the usual elongated pyriform cells, one sees a large number of small spherical ones arranged in distinct chains (Figs. 14, 17). The transition between the two conditions can be traced without much difficulty, in fronds where the cystocarps have just commenced to develop. It is seen that, when the main part of the growth of the plant has taken place, the terminal cells, instead of dividing as frequently vertically, divide more frequently transversely, forming short chains of small cells, which afterwards increase considerably in size. Frequently even in the adult fronds the chains of terminal cells are seen to give place to the ones of the usual form at the base of the frond (Fig. 14). There is nothing remarkable in the appearance of the walls or the contents of these chains of cells that would lead one to suppose that they have any special function. The condition of the cells seems merely to be that which is very often seen in the cystocarps of other algæ, where the outer wall of the conceptacle consists of a great number of small cells in chains. In the case of *C. Polysiphoniæ*, the frond is so small in proportion to the cystocarp that the whole of it becomes modified in this change.

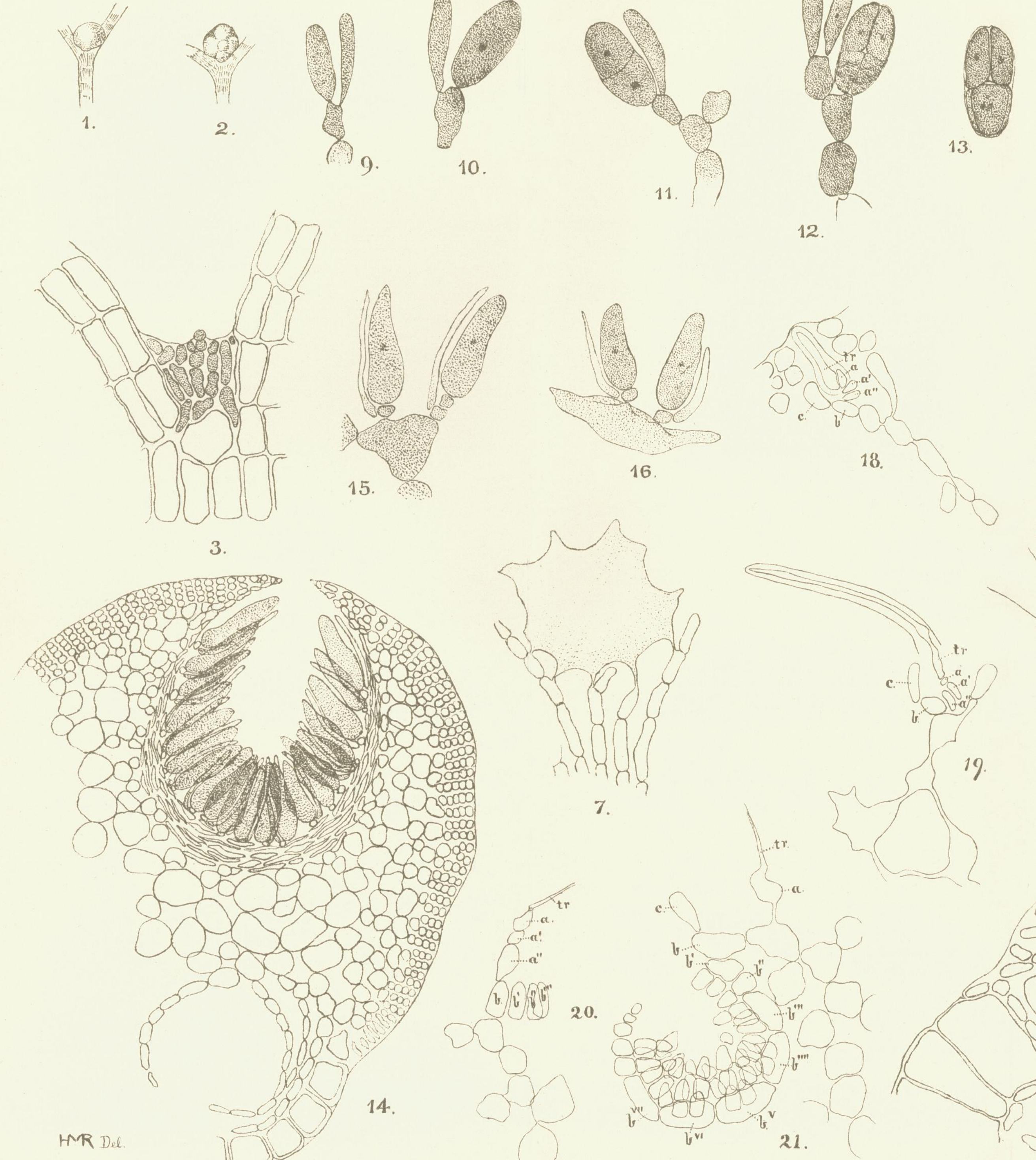
It was not until late in the course of my examination of *C. Polysiphoniæ* that I discovered the trichogyne and its accompanying organs. The fronds containing them were scarched for diligently, but only a few plants were found that were in the right condition. It was undoubtedly too late in the season when I first found the trichogynes to expect them to be common, for then almost all of the cystocarpic fronds were mature. In spite of this, however, sufficient material was found to make out the structure of the undeveloped procarp, and to some extent to follow its development. The trichogyne forms the distal extremity of an irregular chain of cells, which are often connected into a more distinct filament than is common with the interior cells of *C. Polysiphoniæ*. It represents and is developed out of one of a number of cells, which at first were ordinary terminal cells like the others of the frond. The terminal cells arising from the same basal cell as the developing trichogyne and trichophoric apparatus apparently cease all growth after the latter begin to develop, and soon become buried in the frond. One or more of them often remain, as in Figures 18, 19, and 21, *c*. The remaining cell which is to continue the growth divides, the terminal cell developing into the trichogyne, the lower ones forming the trichophoric apparatus. When fully developed, the trichogyne is very long. The cell itself is about 2–2.5 μ in diameter, and often 115 μ long. The diameter of the trichogyne is much greater, however, by reason of a very thick apparently gelatinous sheath of high refrangibility. The whole trichogyne, including sheath, measures 5–6 μ broad. At the apex the sheath becomes much thinner, and consequently does not materially increase the length of the trichogyne. The cell of the trichogyne is often irregular in diameter, frequently exhibiting considerable swellings, which however are not followed by similar swellings in the sheath (Fig. 19). At the base the cell broadens out where it joins the trichophoric apparatus (Figs. 18, 19). The trichogyne usually pierces the cellulose covering without bending or other distortion, and extends for a considerable distance beyond the frond. Not infrequently, however, the trichogynes instead of immediately making their way through the sheath, become bent when they come in contact with its lower surface. They often grow for considerable time between the outer layer of terminal cells and the under surface of the cellulose covering before they succeed in breaking through it. Below the trichogyne are three trichophoric cells. They are usually somewhat wedge-shaped cells, of variable size. The order of their formation I do not know. The one next to the trichogyne is generally smaller than the others, and is set at somewhat of an angle to them (*a*, Figs. 18, 19). In other words, the axis of the procarp curves here. The other two cells of the trichophore lie side by side, and are usually of about the same size (a', a'', Figs. 18, 19). The trichophoric cells are of a decided brownish color, and their contents are quite clear. The cell *b* (Figs. 18, 19), on which the lowest of the trichophoric cells rest, presents very much the appearance of the cells of the rest of the frond. The cells *c* (Fig. 18, 19, 21) I take to be undeveloped terminal cells, previously referred to in the development of the trichogyne. The cell *b*, and apparently in some cases a number of the cells beneath it, are probably the carpogenic cells of the procarp. In all the cases seen it did not seem that the trichophore played any part in the formation of the cystocarp. In Figure 20 the cells a, a', a'', probably correspond to the trichophoric cells indicated by the same letters in the other figures. If this is the case, the fate of the trichophoric cells can be accounted for. After fertilization they shrivel up and finally disappear, without developing further. In the same figure *tr* indicates probable remnants of the trichogyne, while the cells b', b'', and b''' also are the outcome of the division of the original carpogenic cell *b* (Figs. 18, 19). Some cases were observed where the growth of the cystocarp seemed to originate even farther down in the tissue of the plant, but nothing definite was established concerning this. After the stage represented in Figure 20, the development becomes hard to trace. In the next one figured (Fig. 21), the cells *b*, *b'*, *b''*, etc., are probably to be compared with those indicated by the same letters in the previous figure, while *c* is an undeveloped terminal cell before noticed. The cells *b'*, *b''*, etc., have increased enormously in size and in number as well. They have given rise to many smaller cells the fate of which seems probably to be the formation of the cystocarp proper. The course of growth becomes now even more obscure. The cells in the neighborhood of the young cystocarp become complicated in the growth of the wall, and effectually hide the changes which at this time are affecting the cystocarp proper. It seems probable from what was seen that the cells *b*, *b'*, *b''*, etc., start up another growth, and, budding outwards, form, with the surrounding cells whose growth has been already mentioned, the wall of the tabular cells found in the ripe fruit. The small cells first formed from the activity of the cells *b*, *b'*, *b''*, etc., are enclosed in this mass, and develop into the spores and spore-bearing cells. It is to be regretted that the material was so scanty for this work. A search will be made next fall earlier in the season, to find if possible more trichogyne-bearing fronds, and an attempt made to determine more definitely the development of the fruit.

Up to the present time the relationship of *C. Polysiphoniæ* to the rest of the Florideæ has been very uncertain. Heretofore, it will be remembered, nothing but tetraspores has been described. In his list of the Florideæ, Schmitz places it among the Gelidiaceæ with Binderella in the sub-order Binderelleæ. His reason for placing it in the Gelidiaceæ is presumably on account of its general likeness to *Harveyella mirabilis* (Reinsch), Schmitz and Reinke, which in the same list is placed in a separate sub-order, Harveyelleæ, next to the sub-order to which Choreocolax is assigned. Others have followed him in this arrangement, but no one, so far as I know, has placed *C. Polysiphoniæ* in any other order of the Florideæ. In view of what has been described in this paper regarding the structure of the cystocarp, this can scarcely be considered to be its true place. The cystocarp of Harveyella is likened by Schmitz, in his note in Reinke's "Algen Flora der westlichen Ostsee," to that of Caulocanthus, a resemblance which would place Harveyella without doubt among the Gelidiaceæ. It certainly seems impossible to consider the cystocarp of *Choreocolax Polysiphoniæ* as closely related to that found in the Gelidiaceæ. It lacks the most essential feature of similarity to the Gelidiaceæ in the absence of the complicated axial placenta which characterizes that order. The carpospores of *Choreocolax Polysiphoniæ*, it will be remembered, were found to be borne singly all over the inner surface of the cavity, on cells or filaments projecting into its cavity. This fundamental difference in structure certainly makes it impossible to consider *C. Polysiphoniæ* one of the Gelidiaceæ. The condition of the cystocarp approaches far more nearly that found in the Chætangiaceæ than in any other order. I examined cystocarpic specimens of *Chætangium ornatum*, in order to compare them with those of *Choreocolax Polysiphoniæ*. Although the cystocarp of Chætangium is somewhat more complicated than that of Choreocolax, there is a great resemblance between the two. The spores are borne in Chætangium on filaments projecting into the cavity of the cystocarp, much in the same way as was observed in *Choreocolax Polysiphoniæ*. The filaments in Chætangium protrude farther into the cystocarpic cavity than in the other form, but that is not an essential difference. The spores themselves resemble those of *Choreocolax Polysiphoniæ* in shape, but are much smaller. Galaxaura was also examined, and an even closer resemblance seen. The cystocarp of Galaxaura is simple, like that of *Choreocolax Polysiphoniæ*, and the spores are larger than those of Chætangium. The dissimilarity of the fronds found in the various genera of the Chætangiaceæ from that of *Choreocolax Polysiphoniæ* can only be considered as a specific distinction, and not as a valid objection against placing the plant in question in this order. The fronds of the forms already included under the Chætangiaceæ are so dissimilar that there cannot be said to be any typical frond in this order.

Before closing, it seems well to say a few words regarding the methods of work employed in investigating this rather unmanageable alga. The immense amount of gelatinous matter in the frond was of course a most difficult thing to preserve properly. Ordinary methods of killing with corrosive sublimate and chromic and picric acids were tried, and yielded partially satisfactory results. The material thus killed was useful for maceration and dissection. All these reagents, however, caused a great amount of shrinkage in the tissues of the plant. Many other methods that seemed suitable were tried with a hope of finding some reagent that would kill the cells and leave them in a natural condition. Nothing was discovered, however, that served this purpose; in fact, it was found that just as soon as the cells of the plant died, however cautiously they may have been killed, just so soon did they shrink and contract into the grotesque shapes one finds them in. More than this nothing could be found that would swell up the contracted tissues to their lifelike appearance. Potassic hydrate caused a general disintegration, and the various acids, unless used so strong as to dissolve the whole mass, produced but little effect. For this reason, the greater part of the work was done with sections cut between pith, in sea-water, with a razor. Nothing else than sea-water was allowed to touch them, and by this means sections were obtained that would keep alive three or four hours, after which time they gradually contracted as they died. Almost all the drawings were made from these fresh sections, and whatever work was done with dried or alcoholic material was verified by means of them. Many false appearances are given by the immense contraction which takes place when the frond dies, and it was for this reason that these precautions were taken. In order to have a supply of fresh material constantly on hand, I made excursions as often as possible during the winter to Nahant, where *Choreocolax Polysiphoniæ* is fairly abundant. The Choreocolax and the Polysiphonia on which it grew could with care be kept for a considerable time, either in sea-water or moist in a tin box. The latter way is perhaps the better, and if the box is kept moderately cool, and has been well sterilized before putting the material in it, the Choreocolax will keep alive from ten days to two weeks.

In conclusion, I wish to thank all those who have kindly helped me in my work. To Professor W. G. Farlow I am especially obliged, and to Dr. W. A. Setchell I am also indebted for several valuable suggestions.

Cryptogamic Laboratory, Harvard University,
March, 1891.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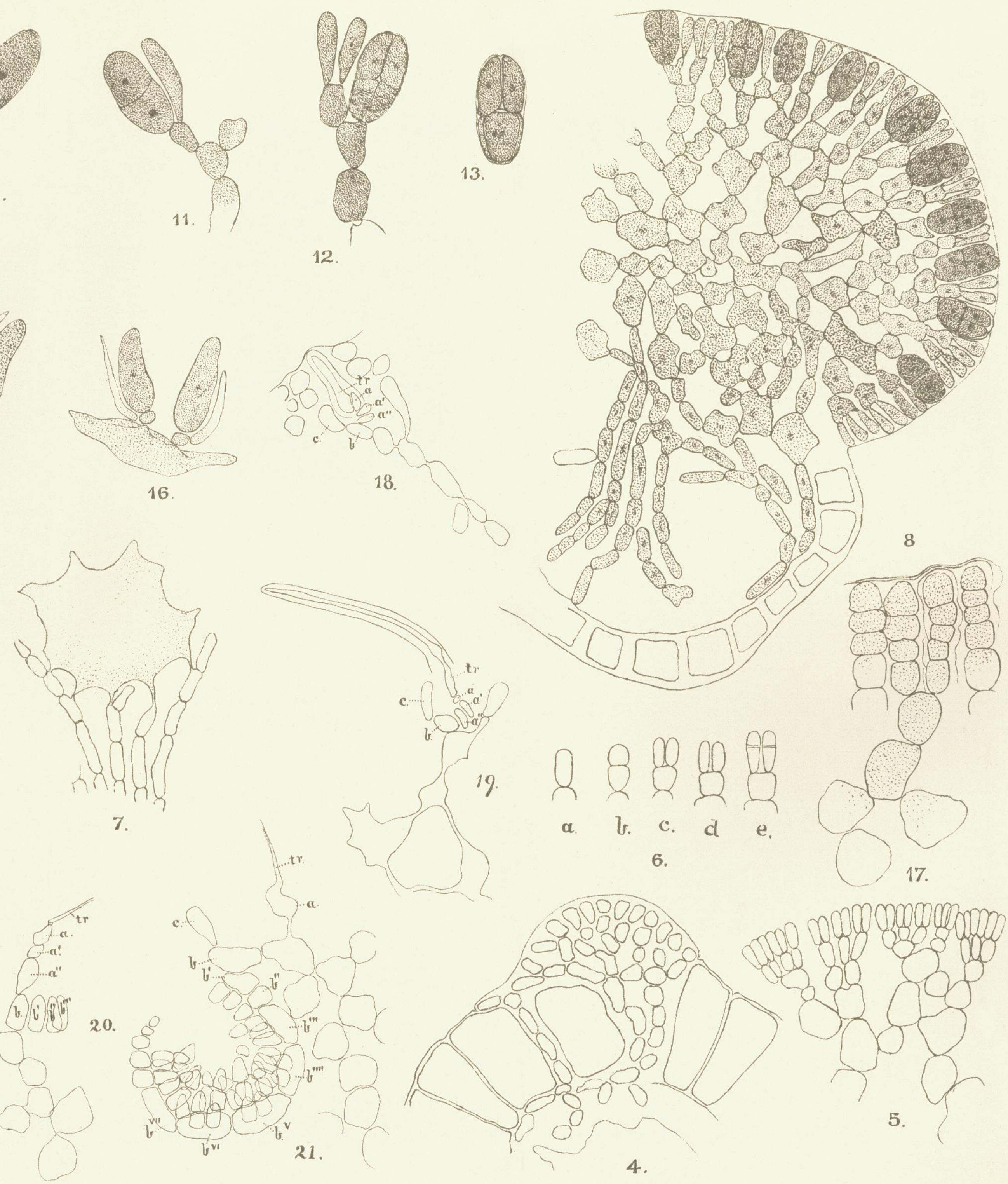

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EXPLANATION OF FIGURES.

Fig. 1. Typical form of frond. × by about 3 diam.
" 2. Lobed cystocarpic frond. × by about 3 diam.
" 3. Very young frond just making its way into the Polysiphonia. × 180.
" 4. Somewhat older stage, where cells are more regularly arranged. × 200.
" 5. Terminal cells of growing frond, from maceration preparation. × 350.
" 6. Diagrams showing method of growth of terminal cells.
" 7. From a preparation shrunk in glycerine to show connection of cells of parasite with host. × 150.
" 8. Vertical section of tetrasporic frond. × 150.
" 9, 10, and 11. Three stages in the development of tetraspore. × 350.
" 12. Adult cruciate tetraspore. × 350.
" 13. Adult tripartite tetraspore. × 350.
" 14. Vertical section of cystocarpic frond. × 150.
" 15 and 16. Showing the two ways in which the carpospores are borne. × 350.
" 17. Portion of periphery of cystocarpic frond, showing chains of cells. × 350.
" 18. Young procarp. × 600.
tr, trichogyne.
a, a,′ a,″ trichophoric cells.
b, carpogenic cell.
c, undeveloped terminal cell.
" 19. Older procarp with fully developed trichogyne. × 600.
References as in Figure 18.
" 20. Procarp in which carpogenic cells have begun to develop. References as in Figure 18, except *b′, b″, b‴*, which are the cells newly formed from the carpogenic cell *b*. × 600.
" 21. More advanced stage in development. References as before. *b⁗, bᵛ*, etc., are the result of further proliferation of cells *b, b′*, etc. × 600.

Figures 1 and 2 were drawn free hand. The others were all drawn with the camera, except Figure 6, which is merely a diagram. Figures 18 to 21 have been reduced one third from the original drawings. All the drawings are from fresh material except Figures 6 and 7, the latter of which was drawn from a section shrunk in glycerine.